

Vaccines



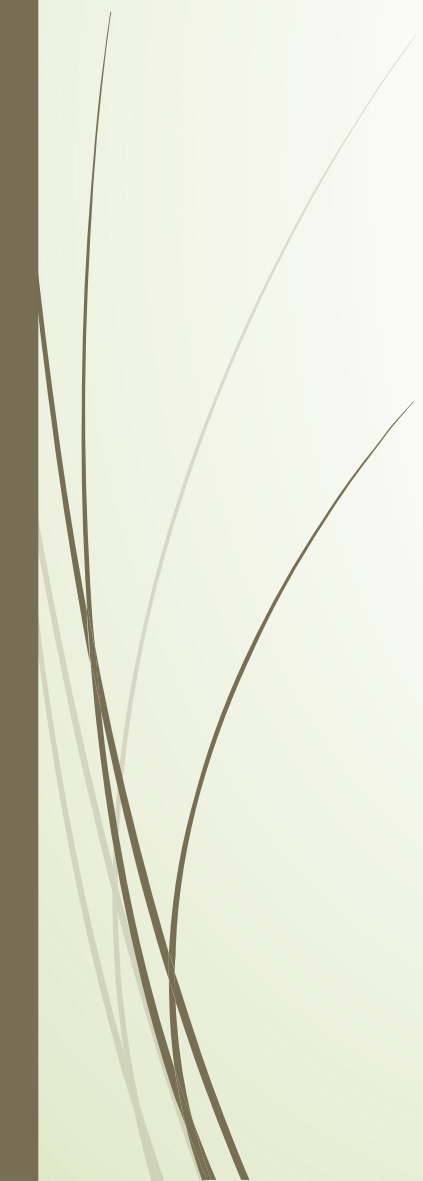
Ahsan Sattar Sheikh, PhD

Associate Professor

Micro/Molecular Biology



Immunization



- **Immunization:** a procedure designed to increase concentrations of antibodies and/or effector T-cells which are reactive against infection (or cancer).
- Immunization procedure called vaccination and the immunizing agent called vaccine (or “serum” in historical references)

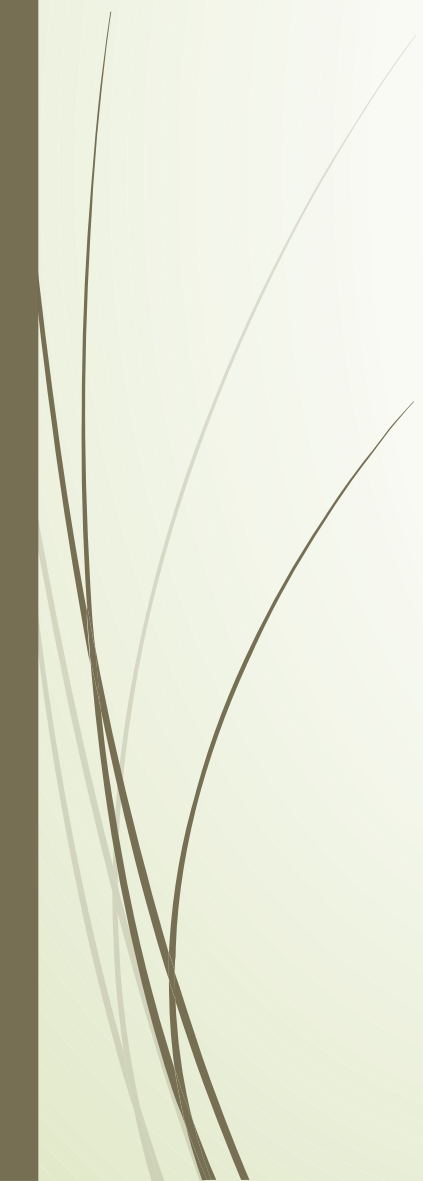


Immunization (cont'd)

- When performed before exposure to an infectious agent (or soon after exposure in certain cases), it is called **immunoprophylaxis**,
- intended to ***prevent*** the infection.
- When performed during an active infection (or existing cancer), it is called **immunotherapy**, intending to ***cure*** the infection (or cancer)



Types of Immunity



- ▮ Two mechanisms by which immunization can be achieved
- ▮ **Passive immunization:**
 - ▮ Protective Abs --> non immune recipient
 - ▮ No immunological memory w/o T_h cells.
- ▮ **Active immunization:**
 - ▮ Induction of adaptive immune response, with protection and memory.



Passive and Active Immunization

TYPE ACQUIRED THROUGH Passive Immunization -

Natural maternal serum/milk

Artificial immune serum

Type ACQUIRED THROUGH Active Immunization -

Natural infection

Artificial infection*:

- Attenuated organisms (live)

- inactivated organisms (dead)

- Cloned genes of microbiological antigens

- Purified microbial macromolecules

- Synthetic peptides

- DNA

**Artificial refers to steps involving human*



Passive Immunity

Naturally - transplacental transfer of maternal IgG Abs to developing fetus; transfer of IgG + IgA Abs in milk during breast-feeding of newborn

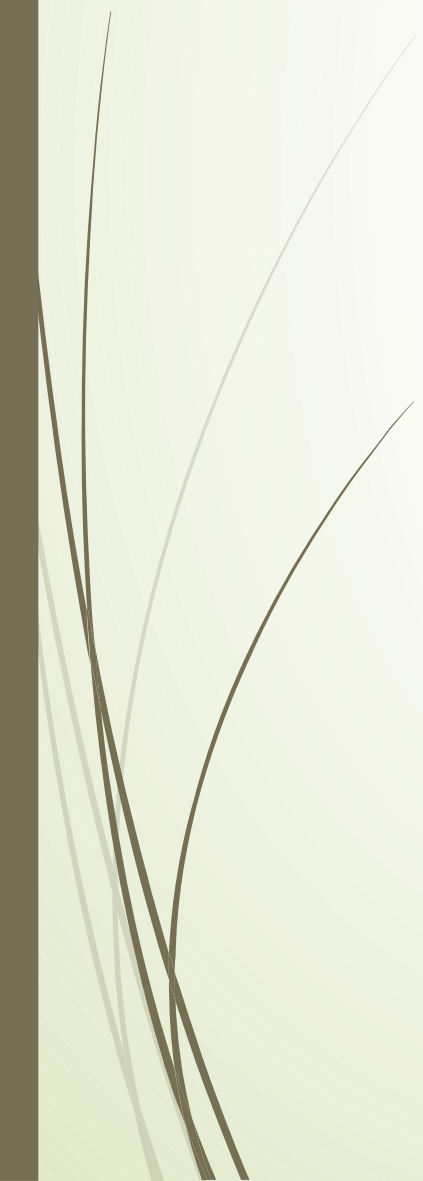
Medically - injection of immune globulin
Performed prophylactically, either after diagnosis of exposure to toxin/virus or as a short term preventive procedure, e.g. if one is traveling to an endemic area

Blocking - prevent hemolytic anemia of the newborn:

Rhogam injected into pregnant Rh- mother prior to



Active Immunization



Naturally - following exposure to an infection

Medically - by vaccination: Performed either by i.m. injection of killed or attenuated antigens (often with adjuvant) or by ingestion of attenuated live organisms.

Blocking - Reversal of auto-immune response

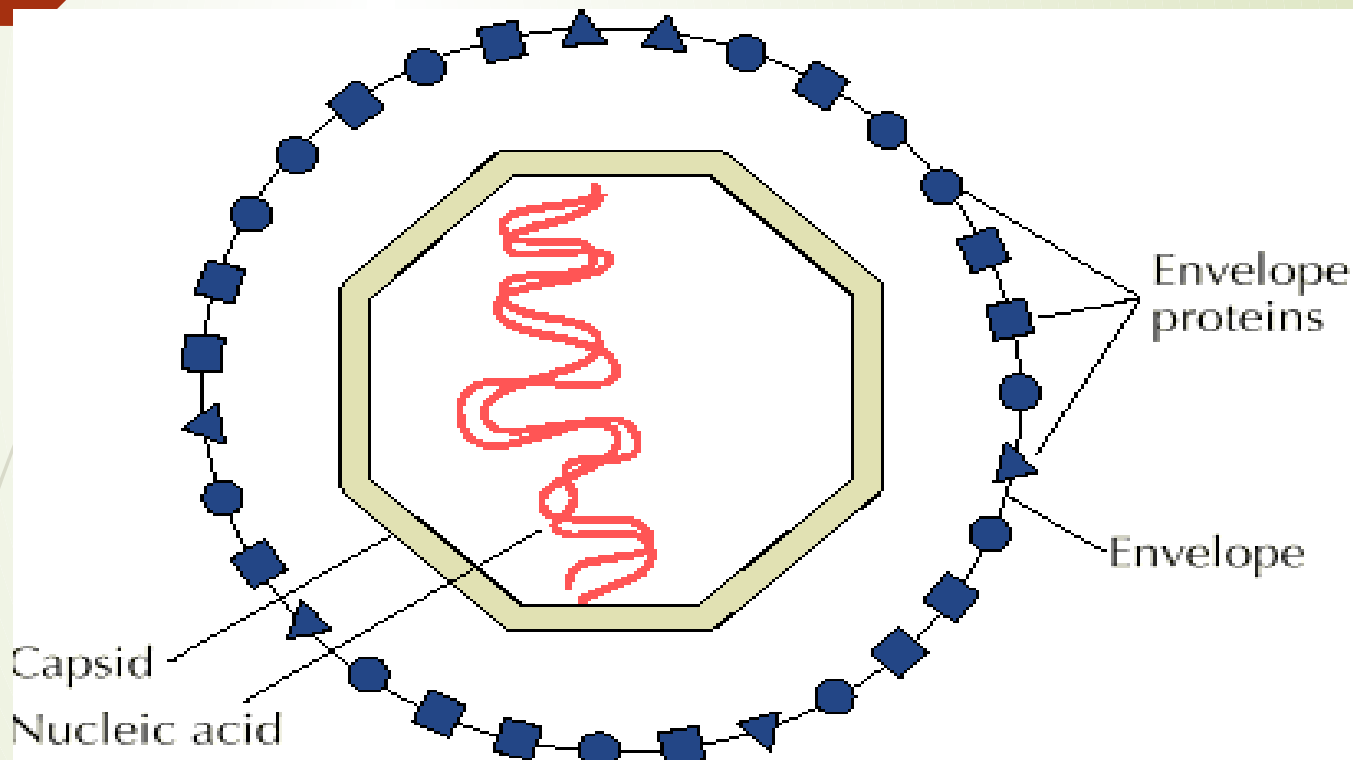
Anti-cancer - Reactivation of tumor-stimulated
T
lymphocytes.



Mechanism of Vaccination

Establish resistance to virus/pathological organism by evoking an immune response

1. Give host a foreign organism/protein in non-infectious form
2. Antibodies are generated
Ab binds to surface proteins of



Structure of a Virus particle

Traditional

I. Types

- A. Inactivated (Killed)
- B. Live
- C. Attenuated (Live, Non-infectious)

LIVE MORE EFFECTIVE THAN
KILLED

II. Pathogens

- A. Bacteria
- B. Virus
- C. Parasites

Types of Vaccines

Attenuated– live microbe (usually virus) which has a vital function inactivated by heat, chemicals or genetic manipulation

- e.g. Rabies virus vaccine, MMR (Measles, Mumps and Rubella)
- BCG (Bacillus Calmette Guerin vaccine for *Mycobacterium tuberculosis*)
- Risk it could revert back to infectious agent
- will stimulate both cell mediated and

Types of Vaccines (cont'd)

- **Inactivated** – uses toxoid – inactivated toxins which are purified proteins
 - stimulates the antibody mediated response only
 - e.g. DPT (diphtheria, pertussis, tetanus toxoids)
 - stimulates the antibody mediated response only

Types of Vaccines (cont'd)

- **Component (subunit)**– contains purified components from bacteria and viruses
- How recombinant viruses are made
 - Hepatitis B vaccine – purified viral coat protein
 - *Streptococcus pneumoniae* (PneumoShot) – capsular polysaccharide
 - *Hemophilus influenzae* (HiB) – capsular polysaccharide, part of DPTPolio-
 - Hib vaccine given to infants
 - *Nisseria meningiditis* – capsular polysaccharide
 - stimulates the antibody mediated response only

Other vaccinations/components

- **Booster Shots:** same vaccine given at a later date (e.g. DT given every 10 years
 - to refresh the memory cell population
- **Adjuvant:** chemicals in the vaccine solution that enhance the immune response
 - Alum – Ag in the vaccine clumps with the alum such that the Ag is released
 - slowly, like a time-release capsule
 - gives more time for memory cells to form



Antibody Titer

- ▮ A test to measures the presence and amount of antibodies in blood against a particular type of tissue, cell, or substance
- ▮ Titer determines if you have adequate protection against a disease
- ▮ May need to give booster if titer too low
- ▮ E.g., happens with HepB vaccine

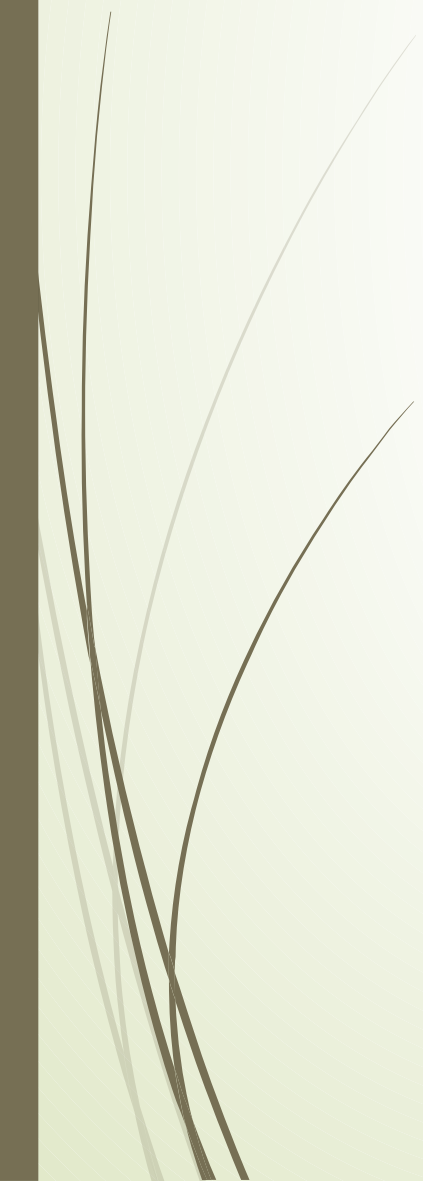


Herd Immunity

- Indirect protection from infection among susceptible members of a population, and the protection of the population as a whole, due to the presence of the immune individuals
- Therefore, leads to reduction of transmission in a population (sometimes can lead to the disappearance of the disease)



Herd Immunity Animation



- ▢ <https://youtu.be/-muloWofsCE>
- ▢ <https://youtu.be/Atrx1P2EkiQ>
- ▢ <https://youtu.be/rb7TVW77ZCs>
- ▢ <https://youtu.be/rAGHXMq9ttw>
- ▢ Immunology Video
- ▢ <https://youtu.be/gKkvTXLvbhs>

Common Vaccinations in Infants and Children

Also See Handout

AGE

Birth--2 mos.

2 mos.

pertussis (DTaP)

conjugate)

2-4 mos.

4 mos.

6-18 mos.

12-15 mos.

4-6 yrs

11-12 yrs

VACCINE

Hepatitis B (recombinant surface Ag)

Diphtheria, tetanus, acellular

Inactivated Polio virus (IPV)

Haemophilus influenzae b (Hib-

Hep B

DTaP, IPV, Hib, PCV

Hep B, DTaP, Polio (IPV), Hib

DTaP, Hib

Varicella zoster/chicken pox (VZV)

Measles, mumps, rubella (MMR)

DTaP, IPV, MMR

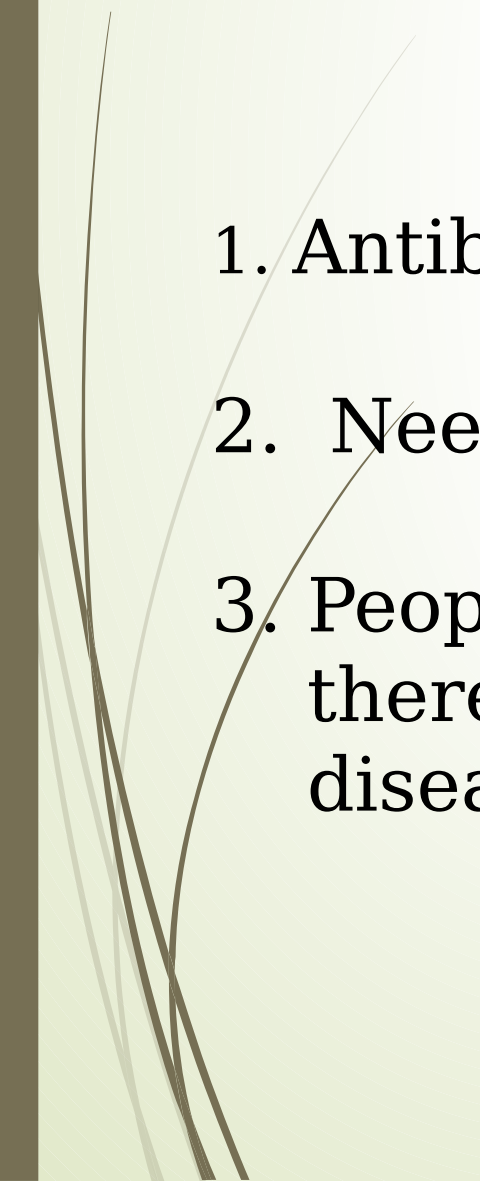
DT

SOURCE: Centers for Disease Control

Limitations To Traditional Vaccines

1. can't grow all organisms in culture
2. safety to lab personnel
3. Expense
4. insufficient attenuation
5. reversion to infectious state
6. need refrigeration
7. do not work for all infectious agents
8. infants/children receive them – immature immunity

Why are antibiotics not good enough?



1. Antibiotic-resistance
2. Need refrigeration
3. People do NOT take entire regiment – therefore better always to prevent disease



Recombinant Vaccines

1. Subunit Vaccines
2. Peptide vaccines
Genetic immunization
3. Attenuated Vaccines
4. Vector Vaccines
5. Bacterial Antigen Delivery Systems

Recombinant Vaccines

1. Delete Virulence Genes (can not revert)
V/B as Vaccine
2. Clone gene for pathogenic antigen into non-pathogenic virus or bacteria
V/B as Vaccine
3. Clone pathogenic antigen gene into expression vector
 - A. Vaccinate with 'protein'
 1. Subunit
 2. Peptide

Subunit vaccines

- Do NOT use entire virus or bacteria (pathogenic agent)
- Use components of pathogenic organism instead of whole organism
- Advantage: no extraneous pathogenic particles ie DNA
- Disadvantage: Is protein same as *in situ*?
Cost

Examples of Subunit Vaccines

A. HSV

- Problem with Traditional vaccine- HSV is oncogenic
- envelope glycoprotein D (gD) elicits Ab response
- Clone gene for gD into vector
- Express in mammalian cells
- Transmembrane protein
modify gene to remove TM portion

Other Subunit Vaccines

B. Tuberculosis

Mycobacterium tuberculosis

antibiotic resistant strains

use purified extracellular (secreted) proteins as Vaccines

C. Foot -and-Mouth Disease virus

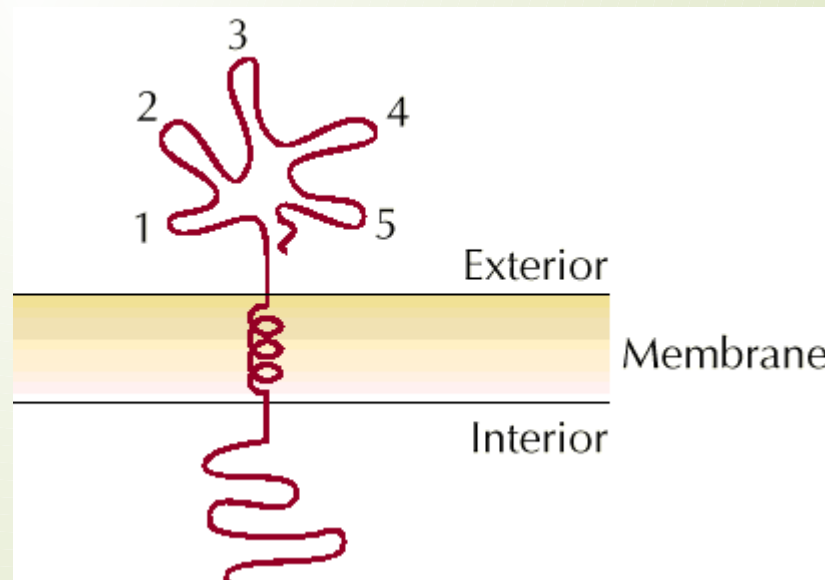
cattle/pigs

VP1 capsid viral protein elicits response -
used this protein as Vaccine

Peptide Vaccines

Use discrete portion (domain) of a surface protein as Vaccine

These domains are 'epitopes'
antigenic determinants
are recognized by antibodies



CARRIER PROTEINS - help vaccine production

Small Peptides are often
Digested

Carrier Proteins Make more
Stable

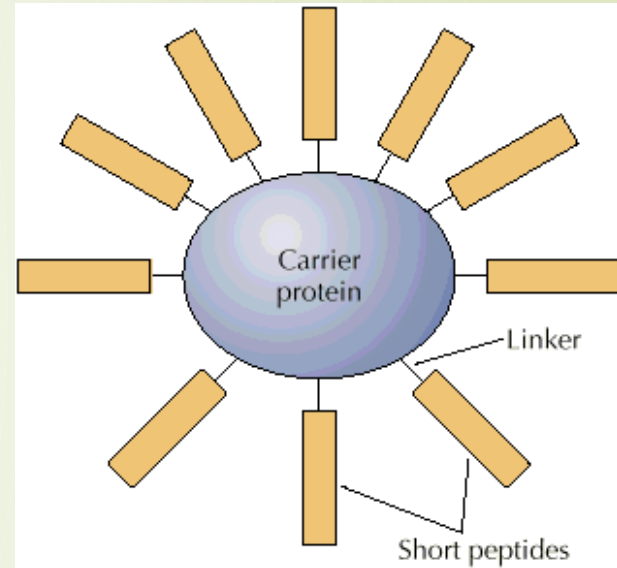
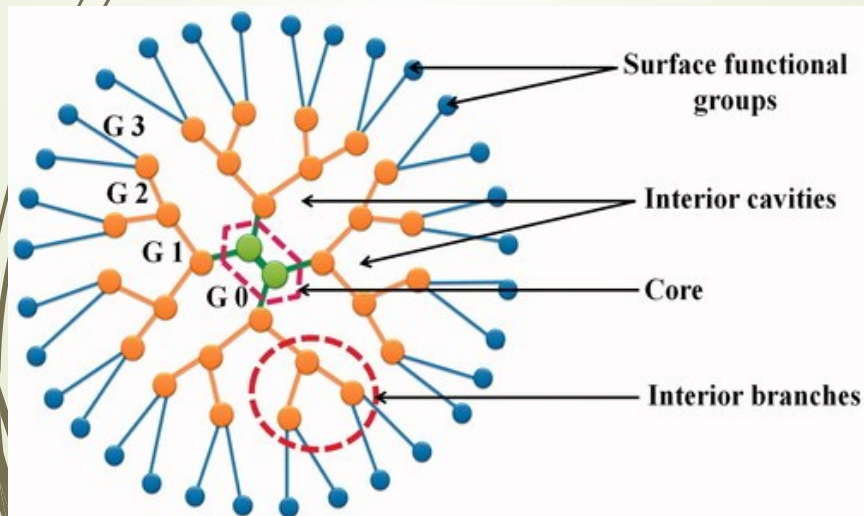


Figure Typical architecture of
dendrimers.





FMDV peptide vaccine

Problem:

Large quantities of peptide needed to be used to get immunological response

Solution:

Use highly immunogenic carrier molecule

HBcAg was a suitable carrier
(Hepatitis Core Protein)

Fused peptide DNA with gene for
HBcAg

Genetic Immunization

Delivery of a gene for the antigen to a host organism

Use vector containing cDNA from viral protein/
eukaryotic promoter

Inject into muscle/microprojectile system

POTENTIAL

eliminates purification of antigen
protein is modified post-
translationally

FATE of plasmid DNA

- integration?
- degradation?

Attenuated vaccines

cholera

caused by bacterium

lives in intestine causing diarrhea, dehydration

poor sanitation (water supply, sewage)

secretes an enterotoxin (A1) which causes disease

killed vaccine not effective long-term

subunit not effective

inactivated cholera used as vaccine currently

typically double deletions are preferred

can not multiply in host

Cholera Vaccines

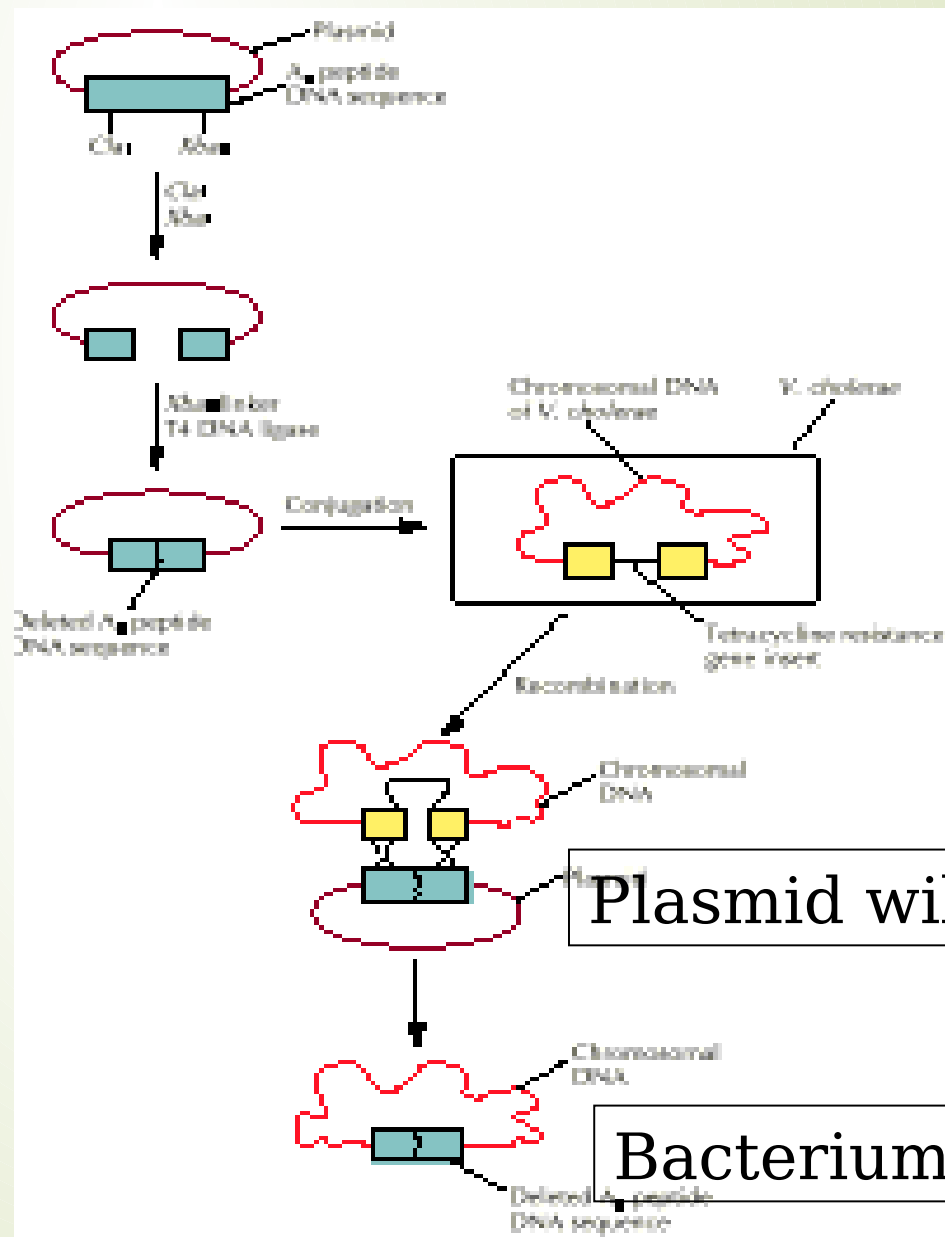
A. Insert tetracycline gene into bacterium's host chromosome
This gene interrupts A1 peptide gene
(toxic portion of the enterotoxin)

NOT ACCEPTABLE

Reversion by spontaneous excision

B. Deleted A1 peptide sequence created

550 bp
removed



Plasmid will eventually be lost

Bacterium will be tetrasensitive

Vector Vaccines: Virus as Antigen Gene Delivery System

Antigen Gene



Virus



Patient

————— Antigen Protein is Made

Vector vaccines

Vaccinia good candidate for a live recombinant viral vaccine

- benign virus
- replicate in cytoplasm (viral replication genes)
- easy to store

A) Insert cloned gene encoding antigen

B) Interrupt thymidine kinase (non-essential gene)

C. Infect host cell with native virus

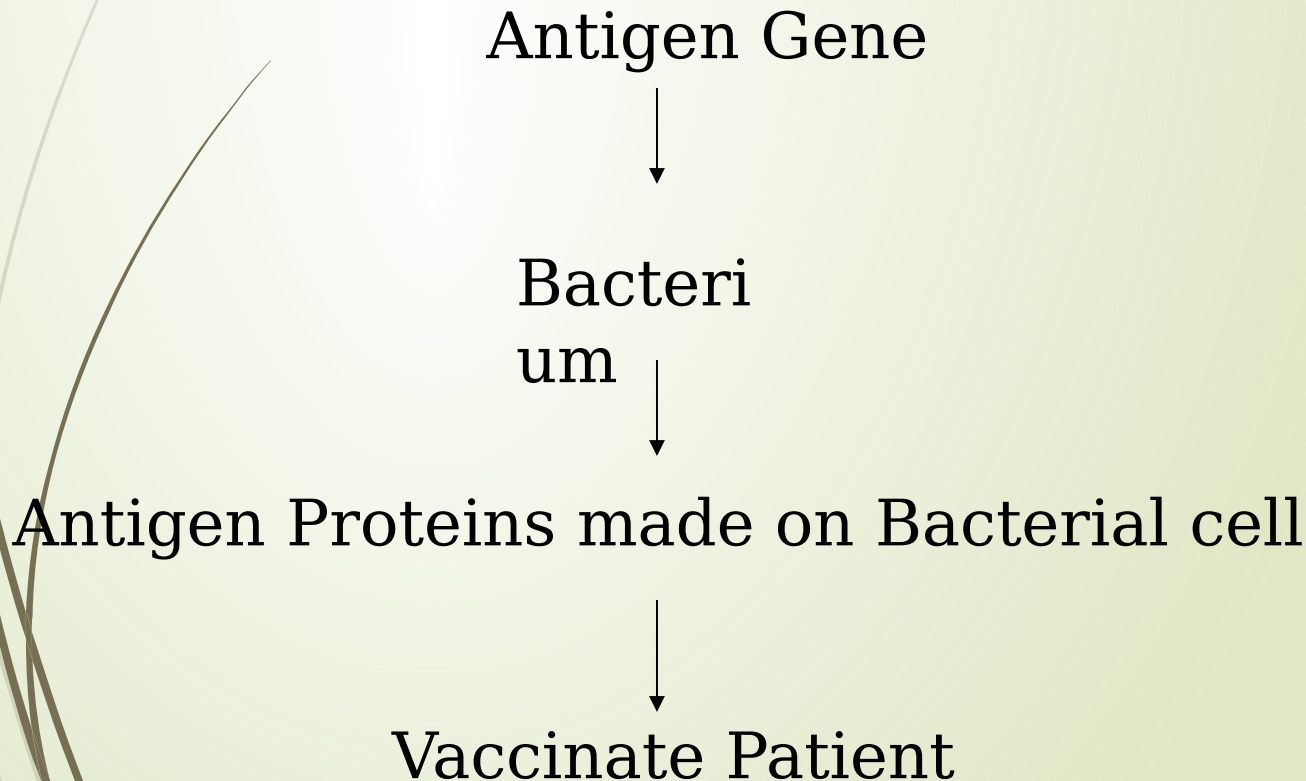
D) Transform these cells with recombinant plasmid

E) HOMOLOGOUS RECOMBINATION

F) Select cells which are resistant to
BROMODEOXYURIDINE

MODIFIED VIRUS USED AS VACCINE

Bacterial Antigen Delivery Systems: Bacterial Vectors



Bacterial Antigen Delivery Systems

Use live nonpathogenic bacterium which contains antigen

(Salmonella) (epitope from cholera)

Insert antigen gene into flagellin gene

Epitope is expressed on the flagellum surface

Flagellin-engineered bacteria is VACCINE**

Advantage - Oral Administration

Does Immunologic Memory = Immune Protection???

- For protection by Abs- sufficiently high level of circulating Abs have to be present during challenge.
- For protection by T cells-a sufficient number of effectors have to be present or have to be generated soon after exposure (consider the pace of infection and presence of Abs)

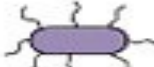
Antigens



Foreign proteins



Viruses



Bacteria



Parasites



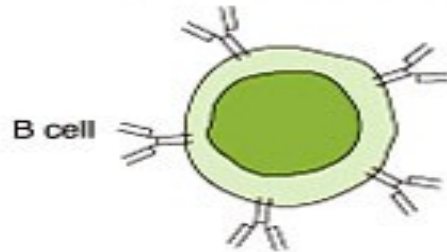
Fungi



Vertebrate body

HUMORAL RESPONSE

CELL-MEDIATED RESPONSE



B cell



T cell

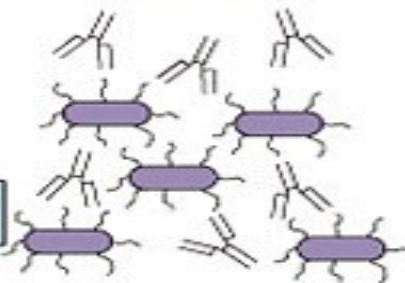


Antigen

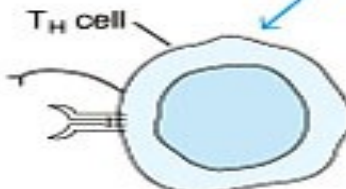
+ T_H-cell cytokines



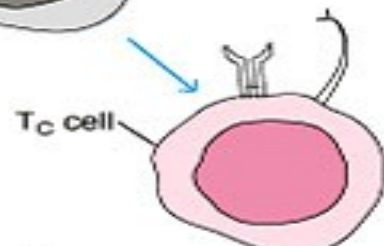
Ab-secreting plasma cells



Antigen elimination



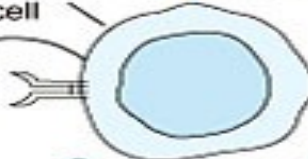
T_H cell



T_C cell

Activated T_H cell

+ Ag—class II MHC molecule



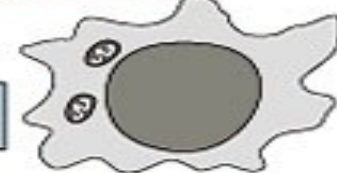
Cytokine secretion



CTL

+ Ag—class I MHC molecule

Killing of altered self-cells



Altered self-cell



“Memories” are made of these

- Increase in frequency of Ag-reactive cells
- Increase in reactivity of effector/memory cells



Vaccine Approval

- Done by CBER (Center for Biologics Evaluation and Research), an arm of the FDA
- Generally same clinical trial evaluation as other biologics and drugs
- Site to learn more about vaccines:

<http://www.fda.gov/cber/vaccine/vacappr.htm>